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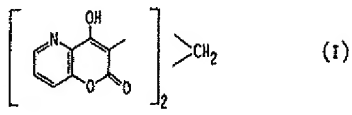
(54) A DERIVATIVE OF 4-HYDROXY-5-AZACOUMARIN

(71) We, A. CHRISTIAENS S. A., a Belgian joint stock company, of 60 Rue de l'Etuve, B-1000 Brussels, Belgium, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a new derivative of 4-hydroxy-5-azacoumarin, and to the preparation of and pharmaceutical compositions containing said new derivative.

According to one feature of the present invention there is provided 3,3'-methylene-bis-(4-hydroxy-5-azacoumarin) which may be represented by the following general formula:



(I)

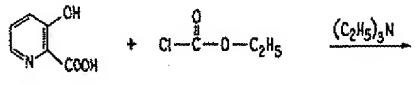
It has been found that the compound of formula (I) has amoebicidal properties, particularly against a human pathogen strain of *Entamoeba histolytica*.

This invention relates therefore also to amebicidal compositions comprising, as active ingredient, said new derivative of 4-hydroxy-5-azacoumarin of formula (I) namely 3,3-methylene-bis-(4-hydroxy-5-azacoumarin), together with a pharmaceutically acceptable carrier or excipient.

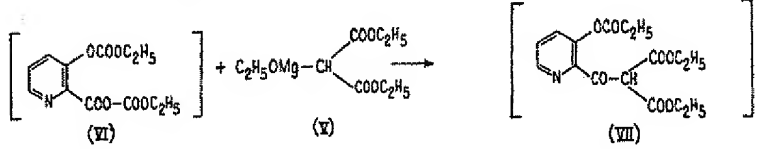
This invention relates also to processes for preparing the new compound of formula (I).

According to this invention, a first process for preparing the new compound of formula (I) involves the following steps:

1) Condensation of *o*-hydroxypicolinic acid (III) successively with ethyl chloroformate (IV) and ethyl ethoxymagnesium malonate (V) or another metal derivative of ethyl malonate, or a metal derivative of ethyl acetylacetate or ethylcyanoacetate, as shown by the following equations:



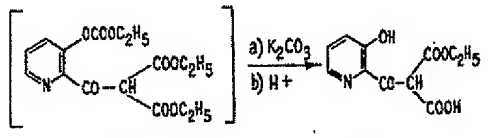
(III)



(VI) (V) (VII)

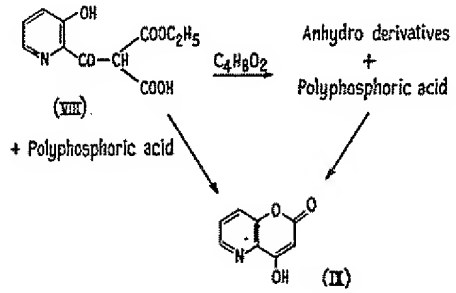
When, in this step 1, ethyl ethoxymagnesium malonate is replaced by a metal derivative of ethyl acetylacetate or ethyl cyanoacetate, an ethyl 3-hydroxypicolinoylacetyl acetate or an ethyl 3-hydroxypicolinoylcyanoacetate is respectively obtained. These latter intermediate compounds are treated in the same manner as intermediate compound (VII) in the following step.

2) Treatment of the obtained intermediate compound (VII) successively with potassium carbonate and with an acid, so as to obtain an hemiester of 3-hydroxypicolinoylmalonic acid (VIII), as shown by the following equation:



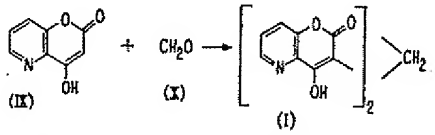
(VII) (VIII)

3) Recrystallization of the hemiester of formula (VIII), for example in dioxan, so as to obtain an anhydro derivative. Cyclization of the ethyl hemiester of formula (VIII) or of the anhydro derivative thereof into 4-hydroxy-5-azacoumarin (IX), for example in the presence of polyphosphoric acid, as shown by the following equations:



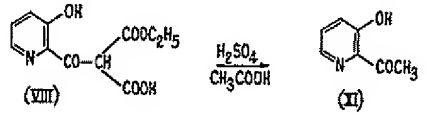
(IX)

4) Treatment of the 4-hydroxy-5-azacoumarin (IX) by means of formaldehyde (X) or a functional derivative thereof in the presence of a polar solvent, as shown by the following equation:



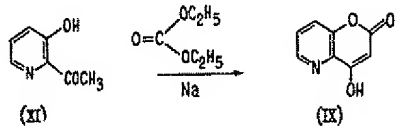
According to this invention, a second process for preparing the new compound of formula (I) involves the following steps:

1) Conversion of the ethyl hemiester of 3-hydroxypicolinoylmalonic acid of formula (VIII) (obtained by steps 1 and 2 of the first process) into 2-acetyl-3-hydroxypyridine (XI) by means of sulfuric acid and acetic acid, as shown by the following equation:

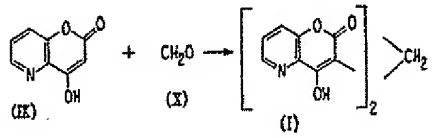


2) Cyclization of the 2-acetyl-3-hydroxypyridine (XI) by means of ethyl

carbonate in the presence of sodium, into 4-hydroxy-5-azacoumarin (IX), as shown by the following equation:

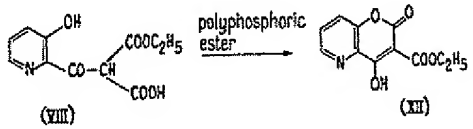


3) Treatment of the 4-hydroxy-5-azacoumarin (IX) by means of formaldehyde (X) or a functional derivative thereof in the presence of a polar solvent, as shown by the following equation:

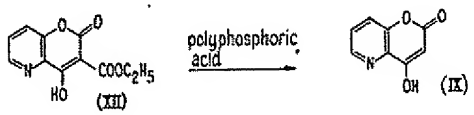


According to this invention, a third process for preparing the new compound of formula (I) involves the following steps:

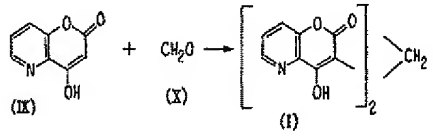
1) Treatment of the ethyl hemiester of 3-hydroxypicolinoylmalonic acid (VIII) (obtained by steps 1 and 2 of the first process) by means of polyphosphoric ester, so as to obtain 3-carbethoxy-4-hydroxy-5-azacoumarin (XII), as shown by the following reaction:



2) Treatment of the 3-carbethoxy-4-hydroxy-5-azacoumarin (XII) with polyphosphoric acid, so as to obtain 4-hydroxy-5-azacoumarin (IX), as shown by the following equation:



3) Treatment of the 4-hydroxy-5-azacoumarin (IX) by means of formaldehyde (X) or a functional derivative thereof in the presence of a polar solvent, as shown by the following equation:



This invention is further illustrated by the following examples:

EXAMPLE 1.

Preparation of the ethyl hemiester of 3-hydroxypicolinoylmalonic acid (formula VIII).

A mixture of 36.5 g (0.26 mol) of *o*-hydroxypicolinic acid, 250 ml of dioxan, 56 g (0.55 mol) of triethylamine and 100 ml of toluene is stirred for 30 minutes in a three-necked flask equipped with a mechanical stirrer, a separating funnel and a drying tube containing calcium chloride.

200 ml of toluene are added to the obtained solution which is then cooled to −5°C by means of a mixture of ice and common salt. Within a period of 30 minutes, a solution of 60 g (0.55 mol) of ethyl chloroformate in 150 ml of toluene is then added to the cooled solution. After maintaining the temperature at 0°C for two hours, a toluene solution of ethyl ethoxymagnesium malonate is added, said solution having been prepared by using 9.72 g (0.4 mol) of magnesium, 28 ml (0.46 mol) of absolute ethanol and 64 g (0.4 mol) of redistilled ethyl malonate.

The obtained solution is stirred overnight and then acidified by adding carefully a solution of 80 ml of 12 N hydrochloric acid in 400 ml of water. The aqueous solution is then stirred for 30 minutes. The organic phase of said solution is then separated by decantation and the aqueous solution is washed two times with benzene. The total organic phases are washed with 0.5 N hydrochloric acid and with water. After drying on sodium sulfate, the solvents are evaporated under reduced pressure. An oily residue (106 g) is obtained.

This residue is added, with stirring, to a heated (80°C) solution of 60 g of potassium carbonate in 300 ml of water. If an oily insoluble material is still present after 4 hours, 4 to 6 g of potassium hydroxide dissolved in a small amount of water are added. When the solution is completely cool, the orange precipitate formed is filtered off. This precipitate is washed with a small quantity of alcohol and then with acetone. The washed precipitate is dissolved in 300 ml of water under gentle heating. The obtained aqueous solution is acidified to a pH of 1—2 by means of hydrochloric acid. An orange precipitate of flakes of the ethyl hemiester of 3-hydroxypicolinoylmalonic acid is obtained. After washing with water and drying, 20.5 g of the desired hemiester are obtained. Melting point: 172—173°C.

Analysis: Found %: C 52.26; H 4.74; N 5.65; mol. weight: 252
Calculated for $C_{11}H_{11}NO_6$ %: C 52.22; H 4.39; N 5.57; mol. weight: 253.195

EXAMPLE 2.

Preparation of the anhydro derivative of the ethyl hemiester of 3-hydroxypicolinoylmalonic acid (formula VIII).

When the product obtained in Example 1 is recrystallized from dioxan, an orange product is obtained. The analysis shows that this product is a dehydration product of the hemiester of Example 1. Said dehydrated product melts at 181—182°C.

Analysis: Found %: C 56.38; H 4.04; N 6.22
Calculated for $C_{11}H_9NO_5$ %: C 56.17; H 3.86; N 5.95

Infrared spectrum: absorption at 1775, 1635, 1485, 1468, 797 and 769 cm^{-1}

N.M.R. spectrum in dimethylsulfoxide at 60°C: $\delta CH_3 = 1$ ppm; $\delta CH_2 = 4.1$ ppm; δH_7 and $\delta H_8 = 8.1$ ppm; $H_6 = 8.6$ ppm.

EXAMPLE 3.

Preparation of 2-acetyl-3-hydroxypyridine (formula XI).

50 g of the ethyl hemiester of 3-hydroxypicolinoylmalonic acid are refluxed on an oil bath for 24 hours with a mixture of 150 ml of acetic acid, 100 ml of water and 20 ml of concentrated sulfuric acid. The cooled mixture is then poured into 600 ml of water and brought to a pH of 5 by addition of sodium bicarbonate, under stirring. The obtained solution is extracted by means of chloroform and the chloroform solution dried on sodium sulfate is concentrated under reduced pressure. The residue is distilled under a vacuum of about 1 mm of Hg. 16 g of the desired product are obtained. After recrystallization from a mixture of methanol and water or from a mixture of acetic acid and water, said product melts at 58°C.

Analysis: Found %: C 61.22; H 4.97; N 10.81
Calculated for $C_7H_7NO_2$ %: C 61.31; H 5.15; N 10.71

EXAMPLE 4.

Preparation of 3-carbethoxy-4-hydroxy-5-azacoumarin (formula XII)

4 g of the ethyl hemiester of 3-hydroxypicolinoylmalonic acid and 50 g of polyphosphoric ester are gently refluxed at 80°C for 1 hour. The cooled solution is

extracted with water and brought under stirring to a pH of about 3 by careful addition of sodium bicarbonate. The stirring is continued for 1 hour, a further amount of sodium bicarbonate being added, if necessary, for maintaining the pH at about 3. The aqueous solution is then extracted two times by 100 ml of chloroform. The chloroform solutions are dried and concentrated under vacuum. The residue is recrystallized from absolute ethanol. 1.1 g of a white product melting at 133°C are obtained.

Analysis: Found %: C 56.28; H 3.73; N 6.09; mol. weight 234.8
Calculated for $C_{11}H_9NO_5$ %: C 56.17; H 3.86; N 5.95; mol. weight: 235.195

EXAMPLE 5.

Preparation of 4-hydroxy-5-azacoumarin (formula IX).

To 2.7 g (0.02 mol) of 3-hydroxy-2-acetyl-pyridine, 24 g (0.2 mol) of ethyl carbonate and 2 g (0.085 mol) of sodium are added. The mixture is then gently heated until the reaction starts and then allowed to stand overnight.

The sodium excess is decomposed by addition of methanol and the solution is then poured into an excess of phosphoric acid solution. The pH is adjusted at a value of 2 and the 4-hydroxy-5-azacoumarin is extracted by means of chloroform. The chloroform solution is dried and concentrated under reduced pressure. The residue is recrystallized from benzene. After a new recrystallization from water or benzene, the product melts at 242°C.

Analysis: Found %: C 58.72; H 3.40; N 8.61; mol. weight: 164.2
Calculated for $C_8H_5NO_3$ %: C 58.90; H 3.09; N 8.56; mol. weight: 163.132

EXAMPLE 6.

Preparation of 4-hydroxy-5-azacoumarin (formula IX).

34 g (0.135 mol) of the ethyl hemiester of 3-hydroxypicolinoylmalonic acid and 210 g of polyphosphoric acid are heated on an oil bath at 127°C until no more gas evolves. After cooling to a temperature of about 30—40°C, the mixture is extracted with 1 litre of water and stirred to dissolve the polyphosphoric acid. 150 g of sodium bicarbonate are slowly added, while stirring, so as to bring the pH of the solution to about 3. The solution is then cooled with water for 30 minutes. The 4-hydroxy-5-azacoumarin precipitates. After filtration and recrystallization from water or benzene, the product melts at 242.5°C (decomposition).

The product may also be recrystallized from a mixture of dioxan and petroleum ether (B.P. 100—140°C) or from absolute alcohol.

Analysis: Found %: C 58.81; H 3.3; N 8.70; mol. weight: 161.9
Calculated for $C_8H_5NO_3$ %: C 58.90; H 3.09; N 8.56; mol. weight: 163.132

EXAMPLE 7.

Preparation of 3,3'-methylene-bis-(4-hydroxy-5-azacoumarin) (formula I).

7 g (0.043 mol) of 4-hydroxy-5-azacoumarin are dissolved in 800 ml of boiling water. 9 ml of a 36% solution of formaldehyde are added and the mixture is left to stand overnight. The precipitate is collected, washed with water and dried at 60°C. 6.4 g of the desired product which decomposes at more than 300°C are obtained.

This product is practically insoluble in water, alcohol, ether, chloroform and benzene. It is soluble in the strong acids and the bases.

Analysis: Found %: C 60.10; H 3.14; N 8.41; equivalent: 166.9
Calculated for $C_{17}H_{10}N_2O_6$ %: C 60.36; H 2.98; N 8.28; equivalent: 169.14

Infrared spectrum: absorptions at 3200 cm^{-1} (OH), 1720 cm^{-1} (C = O), 1642 cm^{-1} and 1582 cm^{-1} (C = C, C = N)

N.M.R. spectrum in CF_3COOH: $\delta H_6 = 8.67$ ppm; $\delta H_7 = 8.05$ ppm; $\delta H_8 = 8.37$ ppm; $\delta CH_2 = 3.81$ ppm.

In vitro and in vivo tests showing the amebicidal activity of 3,3-methylene-bis-(4-hydroxy-5-azacoumarin), will now be described.

In vitro tests:

These tests were performed on the liquid monophase Jones medium inoculated with a human pathogen strain of *Entamoeba histolytica.*

In a first series of tubes, increasing amounts from 6.25 to 100 μg-ml of the compound of formula I (code name JVD 203) were added.

In a second series of tubes, corresponding amounts of a known amebicidal reference compound, i.e. phanquinone (4,7-phenanthroline-5,6-quinone) were added.

A first check tube contained only the culture medium whereas a second check tube contained the same culture medium to which solvents used for solubilizing the active products had been added.

The results of the in vitro tests which were duplicated are summarized in the following table where the signs – and + indicate respectively the absence and the presence of amoebae.

TABLE I.

– Check tubes containing only the culture medium

tube 1 +

tube 2 +

— Check tubes containing the culture medium + solvents

tube 1 +

tube 2 +

— Tubes containing JVD 203 according to this invention

	Concentrations in μg/ml				
	100	50	25	12.5	6.25
tube 1	–	–	–	–	+
tube 2	–	–	–	–	+

— Tubes containing the reference product (phanquinone)

	Concentrations in μg/ml				
	100	50	25	12.5	6.25
tube 1	–	–	–	+	+
tube 2	–	–	–	+	+

These tests clearly show that the product according to this invention (JVD 203) is about two times more active than the reference product.

In vivo tests:

Three weeks old rats of an average weight of 30 g were infested, by intracaecal inoculation of 0.3 ml of a virulent culture of *Entamoeba histolytica.* The animals were divided into 3 groups each comprising 5 animals which were subjected during seven days to one of the three following treatments:

— JVD 203 compound according to this invention: 100 mg/kg/day by oral route,
— Reference compound (phanquinone): 100 mg/kg/day by oral route,
— Physiological solution: 1 ml/10 g/day by oral route.

The animals were killed on the 8th day and the presence of amoebae in the caecum was checked both by microscopic examination and by culture on the

Jones medium. The possible presence of mucus, ulcerations or caecal oedema was also noted.

The results of these in vivo tests are given in the following table II.

TABLE II.

	Rat No.	Ulcerations	Oedema	Mucus	Number of amoebae per microscopic field
Check test (physiological solution)	1	++	++	0	10
	2	++	++	0	5
	3	0	++	0	6
	4	0	++	+	4
	5	0	++	0	8
J.V.D. 203	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
Phanquinone	1	0	+++	0	0
	2	0	+++	0	0
	3	0	+++	0	0
	4	0	+++	0	0
	5	death	+++	0	0

These tests clearly show that the compound according to this invention is at least equivalent and most probably more active than phanquinone as an amebicidal agent.

The following examples illustrate the pharmaceutical compositions according to this invention.

EXAMPLE 8.
TABLET.

3,3'-Methylene-bis-(4-hydroxy-5-azacoumarin)	10 mg
Lactose	200 mg
Potato starch	102 mg
Talc	35 mg
Magnesium stearate	3 mg
for one tablet	

dose: 3 to 6 tablets per day.

EXAMPLE 9.

CAPSULE.

3,3'-Methylene-bis-(4-hydroxy-5-azacoumarin)	100 mg
Lactose	118 mg
Corn starch	60 mg
Colloidal Silica	2 mg
for one capsule	

dose: 2 to 3 capsules per day.

EXAMPLE 10.

PILLS.

Core	
3,3'-Methylene-bis-(4-hydroxy-5-azacoumarin)	50 mg
Lactose	171 mg
Potato starch	13 mg
Polyvidone	13 mg
Talc	3 mg
Coating	
Gum arabic	8.5 mg
Talc	48.5 mg
Cellulose acetophthalate	18.5 mg
Ethyl phthalate	3.9 mg
Magnesium stearate	2,5 mg
Titanium oxide	0.8 mg
Lacquer of orange yellow S	0.2 mg
Sucrose	166.8 mg
White Wax-Carnauba wax	0.3 mg
for one pill	

dose: 1 or 2 pills, three times a day.

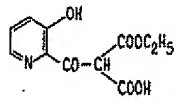
WHAT WE CLAIM IS:—

1. 3,3'-methylene-bis-(4-hydroxy-5-azacoumarin).

2. Amebicidal compositions containing as active ingredient 3,3'-methylene-bis-(4-hydroxy-5-azacoumarin) together with a pharmaceutically acceptable carrier or excipient.

3. A process for preparing 3,3'-methylene-bis-(4-hydroxy-5-azacoumarin) which comprises treating 4-hydroxy-5-azacoumarin with formaldehyde or a functional derivative thereof in the presence of a polar solvent.

4. A process according to claim 3, wherein 4-hydroxy-5-azacoumarin is formed by cyclization of the ethyl hemiester of 3-hydroxypicolinoylmalonic acid of the formula

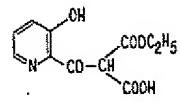


(VIII)

5. A process according to claim 3, wherein 4-hydroxy-5-azacoumarin is formed by cyclization of 2-acetyl-3-hydroxypyridine by means of ethylcarbonate in the presence of sodium.

6. A process according to claim 3, wherein 4-hydroxy-5-azacoumarin is formed by treating 3-carbethoxy-4-hydroxy-5-azacoumarin with polyphosphoric acid.

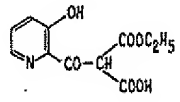
7. A process according to claim 5 wherein 2-acetyl-3-hydroxypyridine is formed by treating the ethyl hemiester of 3-hydroxypicolinoylmalonic acid of formula



(VIII)

with sulfuric acid and acetic acid.

8. A process according to claim 6 wherein 3-carbethoxy-4-hydroxy-5-azacoumarin is formed by treating the ethyl hemiester of 3-hydroxypicolinoyl-malonic acid of formula

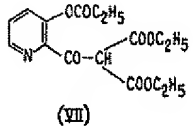


(VIII)

with polyphosphoric ester.

9. A process according to claim 3 wherein 4-hydroxy-5-azacoumarin is prepared by the recrystallization of said hemiester of formula VIII as defined in claim 4 to obtain the corresponding anhydro derivative and then cyclization into 4-hydroxy-5-azacoumarin in the presence of polyphosphoric acid.

10. A process according to any of claims 4, 7 and 8, wherein said ethyl hemiester of 3-hydroxypicolinoylmalonic acid of formula VIII is obtained by condensation of *o*-hydroxypicolinic acid successively with ethyl chloroformate and ethyl ethoxymagnesium malonate to obtain a compound of formula



(VII)

said compound of formula VIi being then treated successively with potassium carbonate and with an acid.

11. A process according to any of claims 4, 7 and 8, wherein said ethyl hemiester of 3-hydroxypicolinoylmalonic acid of formula VIII is obtained by condensation of *o*-hydroxypicolinic acid successively with ethyl chloroformate and a metal derivative of ethyl acetylacetate to obtain 3-hydroxypicolinoylacetyl-

acetate which latter compound is treated successively with potassium carbonate and with an acid.

12. A process according to any of claims 4, 7 and 8 wherein said ethyl hemiester of 3-hydroxypicolinoylmalonic acid of formula VIII is obtained by condensation of *o*-hydroxypicolinic acid successively with ethyl chloroformate and a metal derivative of ethyl cyanoacetate to obtain ethyl 3-hydroxypicolinoyl-cyanoacetate which latter compound is treated successively with potassium carbonate and with an acid.

13. A process for the preparation of 3,3'-methylene-bis-(4-hydroxy-5-azacoumarin) substantially as herein described.

14. A process for the preparation of 3,3'-methylene-bis-(4-hydroxy-5-azacoumarin) substantially as herein described in Example 7.

15. 3,3'-Methylene-bis-(4-hydroxy-5-azacoumarin) whenever prepared by a process as claimed in any of claims 3 to 14.

16. Pharmaceutical compositions as claimed in claim 2, substantially as herein described.

17. Pharmaceutical compositions as claimed in claim 2, substantially as herein described in Examples 8 to 10.

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